SYNTHESIS OF TETRASACCHARIDE CONTAINING GLYCOPEPTIDES RELATED TO BACTERIAL CELL WALL STARTING FROM FREE TETRASACCHARIDE BY THE PENTAFLUOROPHENYL ESTER METHOD*

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The free tetrasaccharide GlcNAc-MurNAc-GlcNAc-MurNAc was converted into the tetrasaccharide bis-pentafluorophenyl ester I by treatment with bis(pentafluorophenyl)carbonate. Reaction of I with suitably protected peptides afforded [GlcNAc-MurNAc-L-Ala-D-Glu(OBzl)$_2$]$_2$ (*II*) and [GlcNAc-MurNAc-L-Ala-D-iGln-L-Lys(Z)-OBzl]$_2$ (*IV*). Catalytic hydrogenolysis of compound *II* and *IV* yielded (GlcNAc-MurNAc-L-Ala-D-Glu)$_2$ (*III*) and (GlcNAc-MurNAc-L--Ala-D-iGln-L-Lys)$_2$ (*V*), respectively. [GlcNAc-MurNAc-L-Ala-D-iGln-L-Lys(St)$_2$]$_2$ (*VI*) was prepared from compound *V* by reaction with stearic acid N-hydroxysuccinimide ester.

Recently, muramyl peptides have aroused special interest as immunomodulators. They possess high biological activity, exhibit no selfantigenicity and toxicity, and their practical application is promising. Some MDP derivatives are recommended as adjuvants[1]. "Muroctasin" (MDP-Lys-L18) can be used against leukopenia in combination with anticancer treatments such as chemotherapy and radiation therapy[2].

Glucosaminylmuramyl peptides are characterized by higher immunological activity than muramyl peptides[3]. In order to expand the list of glycopeptides that serve as models of the bacterial cell wall peptidoglycan, it was of interest to synthesize tetrasaccharide containing glycopeptides. However, such synthesis represents a dif-

* The symbols and abbreviations obey the published recommendations[4]. Other abbreviations: Ac, acetyl; Boc, tert-butyloxycarbonyl; Bzl, benzyl; DMF, N,N-dimethylformamide; GlcNAc, 2-acetamido-2-deoxy-D-glucose; iGln, Glu-NH_2; GMDP, GlcNAc-MurNAc-L-Ala-D-iGln; Lac, lactyl; MurNAc, 2-acetamido-3-O-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucose; Pfp, pentafluorophenyl; St, stearoyl; TFA, trifluoroacetic acid; *Z*, benzyloxycarbonyl. In all oligosaccharides the connection is β-(1 → 4). For the sake of simplicity, tetrasaccharides are denoted (GlcNAc--MurNAc)$_2$.

ficult task. The classical strategy of oligosaccharide preparation is a laborious process, the key reaction of which is the condensation of suitably protected monosaccharides to form the disaccharide[5-8]. This multistep process has special demands on purity and dryness of the reactants and solvents used, and requires many classical column and HPLC purifications. Much more convenient is the preparation of this class of compounds starting from free di- or tetrasaccharide (isolated from *M. lysodeikticus* cell wall after enzymatic digestion)[9-15]. The condensation with suitably protected peptides was carried out using Woodward's reagent K (refs[9-15]). Another approach employs partial protection of the tetrasaccharide with dimethoxytrityl groups and isobutyl chloroformate-induced condensation with the carboxy-protected peptide[11,14,15]. Still better is condensation of the free disaccharide with the peptide by means of bis(pentafluorophenyl)carbonate[16,17]. In this way GMDP was prepared in 60% yield[16]. We tried to apply this method to the synthesis of tetrasaccharide containing muramyl peptides.

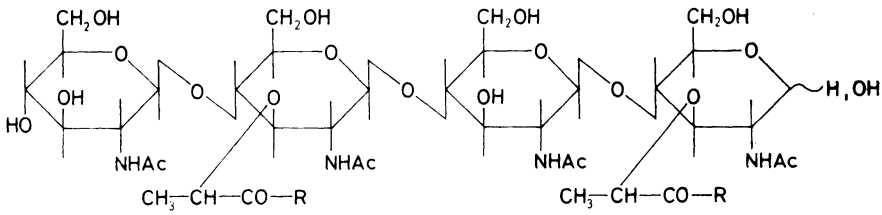
Reaction of free $(GlcNAc\text{-}MurNAc)_2$ with bis(pentafluorophenyl)carbonate in the presence of N-methylmorpholine afforded in 85–94% yield $(GlcNAc\text{-}MurNAc\text{-}OPfp)_2$ (*I*) which was used in condensations with peptide components. Reactions of compound *I* with L-Ala-D-Glu(OBzl)$_2$ (refs[12,18]) and L-Ala-D-iGln-L-Lys(Z)-OBzl (ref.[19]) yielded $[GlcNAc\text{-}MurNAc\text{-}L\text{-}Ala\text{-}D\text{-}Glu(OBzl)_2]_2$ (*II*) and $[GlcNAc\text{-}MurNAc\text{-}L\text{-}Ala\text{-}D\text{-}iGln\text{-}L\text{-}Lys(Z)\text{-}OBzl]_2$ (*IV*), respectively. The crude compound *II* was hydrogenated over palladium black and the resulting $(GlcNAc\text{-}MurNAc\text{-}L\text{-}Ala\text{-}D\text{-}Glu)_2$ (*III*) was purified by preparative HPLC on a Silasorb C18 column and was obtained in 34% yield (29% with correction, see Experimental), in a purity of 93% (HPLC). For biological activity tests, compound *II* was purified on Silica gel 60 (yield 34%, corr. 31%; HPLC purity 92%).

Hydrogenolysis of crude *IV* gave $(GlcNAc\text{-}MurNAc\text{-}L\text{-}Ala\text{-}D\text{-}iGln\text{-}L\text{-}Lys)_2$ (*V*). After desalting on a Sephadex G-25 column, the product *V* was further purified on Bio-Rex 70 (yield 12%, corr. 10%, HPLC purity 90%). HPLC purification of impure fractions from Bio-Rex gave an addional 14% (corr. 12%) of compound *V*, HPLC purity 81%. Direct HPLC purification of the crude desalted glycopeptide *V* is also possible. In this way 23% (corr. 20%) of *V* was obtained in an HPLC purity of 82%.

Reaction of compound *V* with N-hydroxysuccinimide ester of stearic acid, followed by column chromatography on silica gel, afforded $[GlcNAc\text{-}MurNAc\text{-}L\text{-}Ala\text{-}D\text{-}iGln\text{-}L\text{-}Lys(St)]_2$ (*VI*) in 51% yield (corr. 45%) and 85% HPLC purity.

Proton NMR spectra of compounds *III*, *V* and *VI* are generally very complex and their complete analysis is difficult. The reasons are: *a*) the aggregation of molecules in solution resulting in line-broadening; *b*) the existence of α and β anomers in the first (reducing) sugar residue; *c*) the presence of four structurally very similar sugar residues and two peptide moieties.

The aggregation, manifested by line-broadening effects in the spectra of our compounds *III*, *V* and *VI*, depends on the structure, solvent and temperature (Figs 1



I, R = OC_6F_5
II, R = L - Ala - D - Glu(OBzl)$_2$
III, R = L - Ala - D - Glu - OH
IV, R = L - Ala - D - iGln - L - Lys(Z) - OBzl
V, R = L - Ala - D - iGln - L - Lys - OH
VI, R = L - Ala - D - iGln - L - Lys(stearoyl) - OH

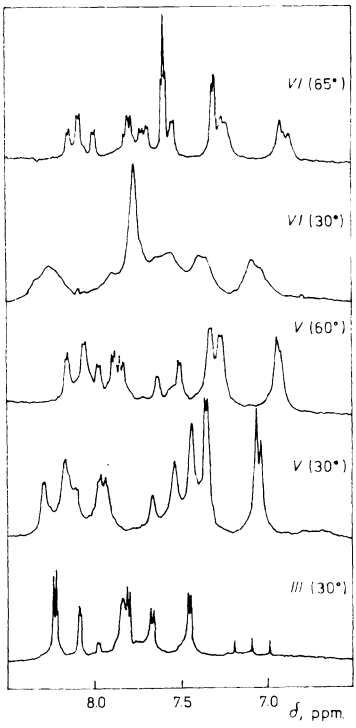


FIG. 1

Proton NMR spectra (NH regions) of compounds *III*, *V* and *VI* in CD_3SOCD_3 at various temperatures

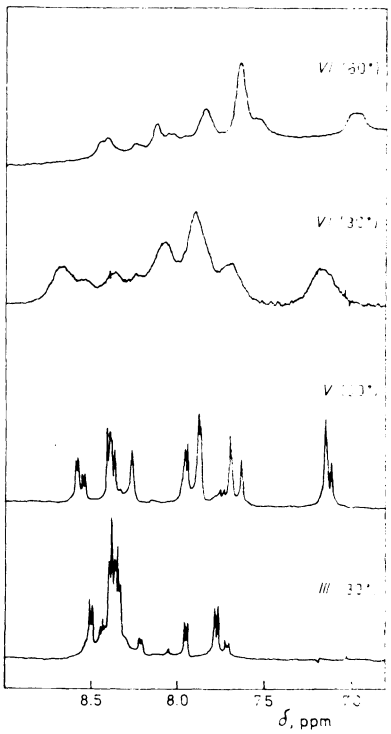


FIG. 2

Proton NMR spectra (NH regions) of compounds *III*, *V* and *VI* in H_2O–D_2O (95 : 5) at various temperatures

and 2). Compound *III* gives rather sharp signals with well resolved multiplets in both H_2O/D_2O and CD_3SOCD_3 already at 30°C. Compound *V* shows a similar behaviour in water solution. On the other hand, in CD_3SOCD_3 solution of the same compound the line-broadening due to the aggregation is evident. The signals become sharper with increasing temperature and well resolved spectrum was obtained at about 65°C. For compound *VI* (containing the stearoyl moiety) we observed extreme line broadening in both solvents at 30°C. Heating to 65°C resulted in only partial signal narrowing for water solution while for CD_3SOCD_3 solution well resolved signals were observed.

The presence of α and β anomers in all the compounds studied gives rise to doubling of some sufficiently sharp signals in the spectrum (e.g. N-acetyl groups) and the ratio of anomers was determined from the relative intensities of corresponding pairs of signals. The α anomer gives a typical downfield shifted signal of anomeric H-1 ($\delta \geqq 5{\cdot}0$) with $J(1, 2) \approx 3$ Hz (ref.[20]) while the same signal of the β anomer is usually overlapped with αCH protons of the amino acid residues in the region δ 3·5–4·5.

Complete identification of all protons of individual sugar residues is nearly impossible (at least from 1D NMR spectra) due to very close chemical shifts and their appearence in heavily overcrowded region together with αCH protons of the amino acid residues. The COSY spectrum of compound *VI* allowed us to assign H-1, H-2, H-3 sequences of all four residues in the major α anomer (see Table I). Two identical peptide side-chains connected to sugar residue 1 and 3 have very similar chemical shift values. As follows from the data of compound *VI* (Table I), their chemical shift nonequivalence decreases with increasing distance from the sugar moiety and it is observable only for the first three residues (Lac, Ala and iGln) while for the others (Lys and St) it disappears. A nearly complete set of NMR parameters for the major α anomer of compound *VI* in CD_3SOCD_3 at 65°C is given in Table I. Partial NMR data for compounds *III* and *V* are summarized in the Experimental.

All the obtained compounds are under study and data on their biological activity will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a Kofler block and are not corrected. Optical activity was measured on a Perkin–Elmer 241 MC polarimeter, the obtained values were corrected according to nitrogen content in the lyophilizate.

Proton NMR spectra of compound *III*, *V* and *VI* were measured on an FT NMR spectrometer Bruker WM-500 (at 500·13 MHz) in: *a*) H_2O and/or and H_2O–D_2O (95 : 5) mixture with water signal supression by selective irradiation; *b*) CD_3SOCD_3. Chemical shifts are given in the ppm (δ scale) and are referenced either to sodium dodecyl sulfate (DSS) or to tetramethylsilane (TMS) as internal standards. The following quantities of samples in 0·4 ml solutions were used: H_2O–D_2O — 4 mg (*III*), 12 mg (*V*) and 5 mg (*VI*); CD_3SOCD_3 — 4 mg (*III*), 5 mg (*V*)

TABLE I

Proton NMR parameters of compound *VI*

Proton		Chemical shifts, (coupling constants, *J*, in Hz)[a]			
		Sugar residue[b]			
		4	3	2	1
	H-1	4·47	4·41	4·44	5·02 (3·0)
	H-2	3·54	3·48	3·71	3·69
	H-3	3·73	3·68	3·55	3·48
NAc:	NH	7·83 (9·0)	7·81 (9·0)	7·73 (8·5)	8·02 (7·0)
	CH_3	1·81	1·82	1·84	1·85
		Non-sugar protons[c]			
Lac:	αCH		4·27		4·38
	βCH_3		1·27		1·30
Ala:	NH		7·56 (6·5)		7·70 (6·0)
	αCH		4·28		4·29
	βCH_3		1·32		1·24
iGln:	NH		8·11 (7·5)		8·16 (7·5)
	αCH		4·21		4·19
	βCH_2		1·82; 2·02		1·79; 1·99
	γCH_2		2·18		2·18
	$CONH_2$		[d]		[d]
Lys:	NH		7·33 (7·0)		7·33 (7·0)
	αCH		4·02		4·02
	βCH_2		1·50; 1·69		1·53; 1·69
	γCH_2		1·28		1·28
	δCH_2		1·37		1·37
	εCH_2		2·99		2·99
	CONH		7·61		7·61
St:	αCH_2		2·04		2·04
	βCH_2		1·48		1·48
	$(CH_2)_n$		1·25		1·25
	CH_3		0·86		0·86

[a] Data obtained in CD_3SOCD_3 at 65°C; only data for the major α-anomer are given; the assignment of protons to individual residues obtained from COSY spectrum; [b] the assignment of data series of residues 2, 3 and 4 is tentative and can be interchanged; signals of sugar protons H-4, H-5, H-6, H-6′ not identified; [c] two series of signals for the Lac, Ala and iGln residues can be interchanged; signals for the Lys and St residues coincide; [d] the value could not be determined.

and 10 mg (*VI*). The spectra were run at different temperatures in the 30°—70°C interval. Typical experimental conditions: spectral width 6 kHz, pulse width 13 μs (flip angle 90°), acquisition time 1·36 s, relaxation delay 0·7 s, number of transients 250 to 1 000, data points 16 K. Phase sensitive 2D-COSY spectrum of compound *VI* was measured in CD_3SOCD_3 at 65°C. FAB Mass spectra were obtained on a Kratos MS 50TC instrument with xenon as gas reagent (gas energy 8 KeV), using glycerol and 1-thio-glycerol matrix.

The purity was checked by TLC on plates of Silica gel 60 (Merck 5724) in the following systems: 1-butanol–acetic acid–water 2 : 1 : 1 (A), chloroform–methanol–water 6 : 4 : 1 (B) and tert-butyl alcohol–DMF–acetic acid–water 2 : 1 : 3 : 2 (C). Detection was effected by ninhydrin or the chlorination method[21,22].

Preparative column chromatography was carried out on silica gel 60 (40—63 μm Merck, 9385). Preparative HPLC separation of crude glycopeptides as well as analyses of the starting mixtures and the obtained fractions were performed on a 344 Beckman chromatographic system. On the preparative scale, detection was made on a 2158 Uvicord SD instrument (LKB, Sweden) at 226 nm, analytical monitoring was made with a 165 variable wavelength detector (Beckman) at 220 nm. Preparative separations were performed on a 250 × 16 mm column packed with Silasorb C18 (particle size 7·5 μm), flow rate 10 ml/min. The preparative column load was 50—100 mg of the crude mixture. Analytical HPLC was carried out on a Ultrasphere ODS column (150 × 4·6 mm, 5 μm), flow rate 1 ml/min. For the stearoyl derivative *VI* a Zorbax TMS column (250 × 4·6 mm, 5 μm) was used.

Yields were expressed mostly by two values, the second one being corrected for the content of the compound in the lyophilizate determined by nitrogen analysis. When only one value is given, it was uncorrected. The glycopeptides were dried over P_2O_5 at room temperature and 10 Pa for 8 to 24 h.

Samples for amino acid analyses were hydrolyzed for 8 h in 6M-HCl at 110°C (ref.[23]) and the hydrolyzates were analyzed on a Durrum D 500 amino acid analyzer. Measurements of standard samples have shown that under the above-mentioned conditions the peak area of muramic acid equals to 60% of the Ala area and the peak area of glucosamine equals to 69% of the Ala area.

The starting $(GlcNAc\text{-}MurNAc)_2$ was obtained from the "Biolar" company (Olaine, U.S.S.R.) (ref.[24]). HPLC purity of this compound was 85% (Ultrasphere ODS; A: TFA solution pH 2·5; B: 30% acetonitrile in A; linear gradient 0 to 100% B during 30 min). Bis(pentafluorophenyl)carbonate[17] was received from the Protein Research Institute of U.S.S.R. Acad. Sci., Pushchino.

$(GlcNAc\text{-}MurNAc\text{-}OPfp)_2$ (*I*)

N-Methylmorpholine (0·83 ml, 7·68 mmol) and $(C_6F_5O)_2CO$ (4·00 g, 10·2 mmol) were added to a stirred solution of free tetrasaccharide (3·74 g, 3·84 mmol) in 12 ml DMF. The reaction was complete in 30 min as indicated by the disappearance of the starting tetrasaccharide (TLC in systems A and B). The product was then precipitated with dry ether (200 ml), filtered, washed with ether (50 ml) and dried. The solid was dissolved in dry methanol (60 ml) at 40°C and precipitated by dry ether (100 ml) and pentane (200 ml). The product was filtered off, washed with ether and dried in vacuo; yield 4·27—4·71 g (85—94%), m.p. 223—227°C. R_F 0·55 in A and B.

$[GlcNAc\text{-}MurNAc\text{-}L\text{-}Ala\text{-}D\text{-}Glu(OBzl)_2]_2$ (*II*)

$Boc\text{-}L\text{-}Ala\text{-}D\text{-}Glu(OBzl)_2$ (refs[12,18]) (0·90 g, 1·8 mmol) was dissolved in 50% TFA solution in methylene chloride (10 ml). After 30 min, the solvents were evaporated in vacuo (40°C) and the evaporation was repeated twice after addition of methylene chloride (10 ml). The residue was triturated with ether, the ether was decanted and the oil was twice coevaporated with methylene

chloride (10 ml). The residue was dissolved in DMF (8 ml) and tetrasaccharide bis-pentafluorophenyl ester *I* (1·18 g, 0·9 mmol) and triethylamine (0·25 ml, 1·8 mmol) were added under stirring. The stirred mixture was kept at pH 7 (wet pH paper) by occasional addition of triethylamine. After 18 h (TLC in systems A and B) ether (50 ml) and pentane (55 ml) were added. The upper phase was decanted and the oily residue was triturated with ether (50 ml). The ether was decanted and the oily residue concentrated in vacuo at 40°C. The residue was dissolved in chloroform–methanol (6 : 3, 10 ml) and purified on a column of silica gel (volume 500 ml, length 60 cm) pre-equilibrated with chloroform. The elution was performed with chloroform–methanol (6 : 3 1 l) and then chloroform–methanol–water 6 : 4 : 1. The product fractions were concentrated, in vacuo, the residue was dissolved in 5% acetic acid and lyophilized. Yield 0·53 g (34%, corr. 31%). Pure according to TLC in systems A (R_f 0·6, ref.[25] 0·63) and B (R_f 0·7). HPLC purity 92%, $[\alpha]_D$ −14·0° (*c* 1; H_2O). For $C_{82}H_{110}N_8O_{33}.CH_3COOH.5H_2O$ (1 885·9) calculated: 53·50% C, 6·63% H, 5·94% N; found: 53·42% C, 6·74% H, 6·17% N. For $C_{82}H_{110}N_8O_{33}$ (1 735·8) calculated: 6·46% N. Glycopeptide content in the lyophilizate 92%. Mass spectrum (FAB): 1 736 (M + 1). Amino acid analysis: Mur 0·99, $GlcNH_2$ 1·13, Ala 0·93, Glu 0·95.

(GlcNAc-MurNAc-L-Ala-D-Glu)$_2$ (*III*)

Crude (GlcNAc-MurNAc-L-Ala-D-Glu(OBzl)$_2$)$_2$ (*II*, prepared from 0·9 mmol of tetrasaccharide bis-pentafluorophenyl ester *I*) was dissolved in a mixture of methanol (20 ml), water (20 ml) and formic acid (2 ml). Palladium black (0·4 g) was added, and the stirred solution was hydrogenolyzed for 6 h (followed by TLC in A and B). Palladium was filtered off and washed with water (3 × 10 ml), the filtrates were concentrated in vacuo, diluted with water and lyophilized. The crude product was purified by preparative HPLC on a Silasorb C18 column (A: TFA solution, pH 2·5; B: 60% acetonitrile in A; linear gradient 6 to 50% B during 50 min). Fractions containing both anomers were collected, concentrated in vacuo and the residues were dissolved in 5%acetic acid and lyophilized. The α : β ratio was 2·34 : 1, yield 0·42 g (34%, corr. 29%, calculated for *I*); pure according to TLC in systems A (R_f 0·25, ref.[25] 0·25) and C (R_f 0·77). HPLC purity 93% (Ultrasphere ODS, A: TFA solution pH 2·5; B: 60% acetonitrile in A; linear gradient 5 to 50% B during 30 min). $[\alpha]_D$ −15·2° (*c* 1·1; H_2O). For $C_{54}H_{86}N_8O_{33}.2CH_3COOH.6H_2O$ (1 603·5) calculated: 43·44% C, 6·66% H, 6·99% N; found: 43·30% C, 6·54% H, 6·84% N. Glycopeptide content in lyophilizate 84%. Mass spectrum (FAB): 1 376 (M + 1). Amino acid analysis: Mur 1·06, $GlcNH_2$ 0·98, Ala 1·00, Glu 0·90. ^{1}H NMR spectrum (H_2O–D_2O; 32°C): purity >95%, ratio of anomers *III*(α)/*III*(β) 2 : 1; 1·32 d (*J* = 7 Hz), 6 H; 1·38 d (*J* = 7 Hz), 3 H; 1·39 d (*J* = 7 Hz), 3 H [4 × CH_3 (Lac, Ala), *III*(α)]; 1·34 d (*J* = 6·8 Hz); 1·37–1·41 [4 × CH_3 (Lac, Ala), *III*(β)]; 1·90 s, 1·92 s, 1·98 s, 2·00 s [4 × NAc, *III*(α)]; 1·89 s, 1·97 s, 1·99–2·00 [4 × NAc, *III*(β)]; 2·05 m, 2·18 m [βCH_2 (Glu)]; 2·43 m [γCH_2 (Glu)]; 3·32–3·97 m (sugar protons); 4·21–4·60 m [αCH (Lac, Ala, Glu) and 3 anomeric protons]; 5·20 d [*J* = 3·5 Hz, H-1 (Mur), *III*(α)]; 7·66–8·60 (NH signals). ^{1}H NMR spectrum (CD_3SOCD_3; 32°C): purity >95%; signals of *III*(α) and *III*(β) not distinguished; δ 1·20–1·40 doublets [*J* = 7 Hz, CH_3 (Lac, Ala)]; 1·75–1·86 singlets (NAc); 1·83 m, 2·00 m [βCH_2 (Glu)]; 2·25 m [γCH_2 (Glu)]; 3·00–3·76 m (sugar protons); 4·17–5·15 [αCH (Lac, Ala, Glu) and anomeric protons].

(GlcNAc-MurNAc-L-Ala-D-iGln-L-Lys)$_2$ (*V*)

Boc-L-Ala-D-iGln-L-Lys(Z)-OBzl (ref.[19]) (1·14 g, 1·71 mmol) was dissolved in 50% TFA solution in methylene chloride (15 ml). After 30 min the solvents were evaporated in vacuo and the evaporation was repeated after addition of methylene chloride (2 × 30 ml). The oily residue was triturated with ether (2 × 30 ml), the ether was decanted and the residue was coevaporated with

methylene chloride (2 × 30 ml). The residue was dissolved in DMF (7 ml) and then compound *I* (1·12 g, 0·86 mmol) and triethylamine (0·24 ml, 1·71 mmol) were added. If necessary, the stirred mixture was adjusted to pH 7—8 (moist pH paper) by addition of triethylamine. After 18 h ether (30 ml) and hexane (30 ml) were added. The upper phase was decanted and the oily residue was triturated with ether (50 ml). The ether was decanted and the residue was concentrated in vacuo and dissolved in a mixture of methanol (30 ml), acetic acid (30 ml) and water (20 ml). The stirred solution was hydrogenated over palladium black (0·5 g) under atmospheric pressure for 6 h (followed by TLC in systems A and B). The catalyst was filtered off, washed with water, the filtrate was concentrated in vacuo, diluted with water and lyophilized. The lyophilizate was dissolved in water (10 ml) and 5 ml aliquotes were used for purification on a Sephadex G-25SF column (volume 425 ml, length 80 cm), equilibrated with 1% acetic acid. The purification was monitored at 226 nm and 10 ml fractions were collected. The product was eluted in the second peak (fractions 22—29). Evaporation in vacuo and lyophilization afforded 1·07 g (77%, calculated to *I*) of compound *V*. Further purification was carried out on a Bio-Rex 70 (Bio Rad, U.S.A.) column (H^+-form, 80 ml, length 23 cm). The column was eluted first with water (200 ml) and then with acetic acid (using a slow gradient from 0 to 0·3 mol l^{-1}). Fractions of the pure product were evaporated in vacuo and lyophilized, yield 0·167 g (12%, corr. 10%, calculated to *I*), pure according to TLC in system C (R_F 0·5), HPLC purity 90% (Ultrasphere ODS, A: 0·02M ammonium acetate, pH 6·5; B: 50% acetonitrile in A; gradient 5 to 100% B during 50 min). Other impure fractions containing product were purified by preparative HPLC on a Silasorb C18 column (A: 0·02M ammonium acetate, pH 6·5, B: 50% acetonitrile in A; gradient 5 to 50%B during 60 min). Lyophilization and desalting on a Sephadex G-25SF column furnished another 0·20 g (14%, corr. 12%) of lyophilizate; HPLC purity 81%. This material was used in the preparation of the stearoyl derivative *VI*. For biological activity tests, the 90% pure product (0·167 g) was purified by preparative HPLC. In this way 0·117 g (8·4%, corr. 7·2%) of compound *V* was obtained; HPLC purity 98%. It was also possible to purify directly the crude product after the chromatography on Sephadex G-25 by means of preparative HPLC; yield 0·321 g (23%, corr. 20%), HPLC purity 82%. In this case, the HPLC purification was complicated by the presence of impurities with mobilities between those of the α and β anomers of *V*. $[\alpha]_D$ −18·4° (*c* 1·0; H_2O). For $C_{66}H_{112}N_{14}O_{33} . 0{\cdot}5\ CH_3COOH.15H_2O$ (1 929·9) calculated: 41·70% C, 7·52% H, 10·16% N; found: 41·60% C, 7·38% H, 10·30% N. The glycopeptide content in the lyophilizate was 86%. Mass spectrum (FAB): 1 630 (M + 1). Amino acid analysis: Mur 1·04, $GlcNH_2$ 0·99, Ala 1·00, Glu 0·96, Lys 1·01. ^1H NMR spectrum (H_2O–D_2O; 32°C): ratio of anomers *V*(α)/*V*(β) 2 : 1: values for both anomers, unless specified; 1·28—1·46 doublets [CH_3 (Lac, Ala)]; 1·66 m, 8 H; 1·77 m, 4 H [βCH_2, γCH_2, δCH_2 (Lys)]; 1·926 s, 1·935 s, 2·016 s, 2·024 s [4 × NAc, *V*(α)]; 2·13 m, 2·20 m [βCH_2 (iGln)]; 2·37 m [γCH_2 (iGln)]; 2·98 t [εCH_2 (Lys)]; 3·30—5·00 [αCH (Lac, Ala, iGln, Lys) and sugar protons]; 5·21 d [*J* = 3·1 Hz, H-1 (Mur), *V*(α)]; 7·10 s, 7·62 s [$CONH_2$ (iGln), *V*(β)]; 7·14 s, 7·69 s [$CONH_2$ (iGln), *V*(α)]; 7·68—8·60 (NH signals). ^1H NMR (CD_3SOCD_3; 30°C): very broad signals; 1·15—1·40 m [CH_3 (Lac, Ala)]; 1·53 m, 1·66 m [βCH_2, γCH_2, δCH_2 (Lys)]; 1·79 s, 3 H, 1·83 s, 6 H ,1·90 s, 3 H (4 × NAc); 1·82 m, 1·97 m [βCH_2 (iGln)]; 2·13 m [γCH_2 (iGln)]; 2·73 t [εCH_2 (Lys)]; 2·95—4·55 [αCH (Lac, Ala, iGln, Lys) and sugar protons]; 4·97 b [H-1 (Mur), *V*(α); at 60°C it gives a doublet with *J* = 3·2 Hz]; 7·03 s, 7·06 s, 7·33 s, 7·35 s [$CONH_2$ (iGln)]; 7·38—8·33 (NH signals).

[GlcNAc-MurNAc-L-Ala-D-iGln-L-Lys(St)]$_2$ (*VI*)

Triethylamine (56 μl, 0·4 mmol) was added to a suspension of compound *V* (0·38 g, 0·2 mmol corresponding to 86% of glycopeptide in the lyophilizate) and stearic acid N-hydroxysuccinimide ester[26] (0·23 g, 0·6 mmol) in DMF (6 ml). After vigorous stirring for 24 h, the obtained solution

was concentrated to dryness in vacuo (oil pump). The residue was dissolved in a mixture chloroform–methanol–water 6 : 4 : 1 (8 ml) and purified on a column of silica gel (320 ml, length 60 cm), pre-equilibrated with chloroform; elution with chloroform–methanol–water (6 : 4 : 1). Product containing fractions were concentrated in vacuo, dissolved in 5% acetic acid and lyophilized; yield: 0·22 g (51%, corr. 45%). Pure according to TLC in systems A (R_F 0·58) and B (R_F 0·41). HPLC on a Ultrasphere ODS column was not successful owing to high retention of compound *VI* on the stationary phase. Analytical HPLC was performed on Zorbax TMS (A: 0·1% solution of TFA; B: acetonitrile; gradient 5 to 80% B during 40 min), HPLC purity 85%, $[\alpha]_D$ −12·0° (*c* 1·0; methanol). For $C_{102}H_{180}N_{14}O_{35}.3CH_3COOH.6H_2O$ (2 450·9) calculated: 52·93% C, 8·39% H, 8·00% N; found: 52·75% C, 8·21% H, 7·98% N. For $C_{102}H_{180}N_{14}O_{35}$ (2 162·6) calculated: 9·07% N. Content: 88% of the glycopeptide in the lyophilizate. Mass spectrum (FAB): 2 162 (M + 1). Amino acid analysis: Mur 1·02, $GlcNH_2$ 1·00, Ala 1·00, Glu 0·93, Lys 0·83. ^{1}H NMR spectrum (H_2O–D_2O; 30°C): very broad signals; 0·84 [CH_3 (St)]; 1·25 [$(CH_2)_n$ (St) and CH_3 (Lac, Ala)]; 1·56–1·75 [βCH_2, γCH_2, δCH_2 (Lys)]; 1·95, 1·97, 2·03, 2·04 (4 × NAc); 2·19 [αCH_2 (St)]; 2·38 [γCH_2 (iGln)]; 3·13 [εCH_2 (Lys)]; 3·30–5·00 [αCH (Lac, Ala, iGln, Lys) and sugar protons]; 6·90–8·90 (NH signals); at 60°C anomeric proton of compound *VI*(α) resolved (5·21 d; J = 3·2 Hz). ^{1}H NMR spectrum (CD_3SOCD_3; 30°C): very broad signals; 0·81 [CH_3 (St)]; 1·20 [$(CH_2)_n$ (St) and CH_3 (Lac, Ala)]; 1·33–1·43 [βCH_2, γCH_2, δCH_2 (Lys)]; 1·79, 1·82 (NAc); 2·00 [αCH_2 (St) and βCH_2 (iGln)]; 2·14 [γCH_2 (iGln)]; 2·95 [εCH_2 (Lys)]; 3·10–4·50 [sugar protons and αCH (Lac, Ala, Lys, iGln)]; 5·02 [anomeric proton of *VI*(α)]; 6·60–8·60 (NH signals). For complete set of NMR parameters of compound *VI*(α) at 65°C see Table I.

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